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GENOME ANNOUNCEMENT

Genome Sequence of *Pichia kudriavzevii* M12, a Potential Producer of Bioethanol and Phytase

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A draft genome sequence of *Pichia kudriavzevii* M12 is presented here. The genome reveals the presence of genes encoding enzymes involved in xylose utilization and the pentose phosphate pathway for bioethanol production. Strain M12 is also a potential producer of phytases, enzymes useful in food processing and agriculture.

Pichia kudriavzevii is synonymously known as *Issatchenkia orientalis* and is an anamorph of *Candida krusei* (2, 8). *P. kudriavzevii* has been isolated from food and fruit sources, such as sourdoughs (11), fermented butter-like products (13), the starter culture of Tanzanian fermented togwa (12), the African fermented cassava lafun (14), a Ghanaian fermented cocoa bean heap (5), fermented pineapple juice (3), orange juice (1), and grape (18). *P. kudriavzevii* M12 was isolated from a local mixed culture of effective microorganism (EM), and its biotechnological potential remains unexplored. The draft genome of strain M12 was determined and annotated in order to gain further insight into the properties of the yeast, including the metabolism pathways of commercial interest.

The draft genome sequence of *P. kudriavzevii* M12 was determined using the Genome Analyzer IIx (100-bp paired-end read). The reads were assembled *de novo* into 626 contigs (175-fold coverage) using CLC Genomics Workbench 4.8 (CLC bio, Denmark). The draft genome contains approximately 10,448,518 bp and has a GC content of 38.32%. The N_{50} is 40,913 bp, and the longest contig is 192,239 bp. The open reading frames were predicted using GeneMark-ES (16) and subsequently annotated by Blast2GO (4). A total of 4,863 protein-encoding genes were predicted, and 480 of the genes have no similarity to current public database sequences. Strain M12 possesses a copy each of the 8S, 28S, and 18S rRNA genes, which were predicted using RNAmmer (9). In addition, there were 145 tRNAs, including 4 pseudo-tRNAs, as analyzed using tRNAscan-SE (10).

The draft genome revealed the commercial potential of *P. kudriavzevii* M12 as a strain of xylose-fermenting yeast for ethanol production. Strain M12 possesses the genes coding for xylose reductase, xylitol dehydrogenase, and xylulokinase responsible for the two-step conversion of xylose to D-xylulose-5-P, which is subsequently channeled into the pentose phosphate pathway for ethanol production.

In addition, the draft genome of *P. kudriavzevii* M12 reveals the presence of 3 genes that code for phytases. This class of enzymes has significant importance in the improvement of phytate dephosphorylation in the processing and manufacturing of food for both human and animal consumption (6). In the application of strain M12 as an EM biofertilizer, the yeast may secrete phytase that degrades phytate in soil and may improve acquisition of phosphorus by plants, thus reducing the need for phosphorus fertilizer (15). Though phytases from *P. kudriavzevii* TY13 and *P. anomala* have been reported (7, 17), there has never been any gene isolation and database deposit of the phytase gene from *Pichia* species. The presence of phytase genes in this genome could open up opportunities for future molecular cloning and application studies of phytase from *P. kudriavzevii* M12.

Nucleotide sequence accession number. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession ALNQ00000000. The version described in this paper is the first version, ALNQ01000000.

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REFERENCES

1. **Arias CR, Burns JK, Friedrich LM, Goodrich RM, Parish ME.** 2002. Yeast species associated with orange juice: evaluation of different identification methods. Appl. Environ. Microbiol. **68**:1955–1961.
2. **Carlotti A, Couble A, Domingo J, Miroy K, Villard J.** 1996. Species-specific identification of *Candida krusei* by hybridization with the CkF1,2 DNA probe. J. Clin. Microbiol. **34**:1726–1731.
3. **Chanprasartsuk OO, Prakitchaiwattana C, Sanguandeekul R, Fleet GH.** 2010. Autochthonous yeasts associated with mature pineapple fruits, freshly crushed juice and their ferments; and the chemical changes during natural fermentation. Bioresour. Technol. **101**:7500–7509.
4. **Conesa A, et al.** 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics **21**: 3674–3676.
5. **Daniel HM, et al.** 2009. Yeast diversity of Ghanaian cocoa bean heap fermentations. FEMS Yeast Res. **9**:774–783.
6. **Greiner R, Konietzny U.** 2006. Phytase for food application. Food Technol. Biotechnol. **44**:125–140.
7. **Hellstrom AM, Almgren A, Carlsson NG, Svanberg U, Andlid TA.** 2012. Degradation of phytate by *Pichia kudriavzevii* TY13 and *Hanseniaspora guilliermondii* TY14 in Tanzanian togwa. Int. J. Food Microbiol. **153**:73–77.
8. **Kurtzman CP, Smiley MJ, Johnson CJ.** 1980. Emendation of the genus *Issatchenkia* Kudriavzev and comparison of species by deoxyribonucleic acid reassociation, mating reaction, and ascospore ultrastructure. Int. J. Syst. Bacteriol. **30**:503–513.

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9. **Lagesen K, et al.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. **35**:3100–3108.
10. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**: 955–964.
11. **Meroth CB, Hammes WP, Hertel C.** 2003. Identification and population dynamics of yeasts in sourdough fermentation processes by PCR-denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. **69**:7453–7461.
12. **Mugula JK, Narvhus JA, Sørhaug T.** 2003. Use of starter cultures of lactic acid bacteria and yeasts in the preparation of *togwa*, a Tanzanian fermented food. Int. J. Food Microbiol. **83**:307–318.
13. **Ongol MP, Asano K.** 2009. Main microorganisms involved in the fermentation of Ugandan ghee. Int. J. Food Microbiol. **133**:286–291.
14. **Padonou SW, et al.** 2009. The microbiota of Lafun, an African traditional cassava food product. Int. J. Food Microbiol. **133**:22–30.
15. **Richardson AE, Hadobas PA, Hayes JE.** 2001. Extracellular secretion of *Aspergillus* phytase from *Arabidopsis* roots enables plants to obtain phosphorus from phytate. Plant J. **25**:641–649.
16. **Ter-Hovhannisyan V, Lomsadze A, Chernoff YO, Borodovsky M.** 2008. Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training. Genome Res. **18**:1979–1990.
17. **Vohra A, Satyanarayana T.** 2002. Purification and characterization of a thermostable and acid-stable phytase from *Pichia anomala*. World J. Microbiol. Biotechnol. **18**:687–691.
18. **Zott K, et al.** 2010. Characterization of the yeast ecosystem in grape must and wine using real-time PCR. Food Microbiol. **27**:559–567.